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(54) Keratin polymer containing
S-sulphocysteine residues; process for
its preparation and the compositions
containing it

(57) A keratin product, which can be
used for cosmetic purposes, comprises
keratin peptide chains of different
molecular weights, formed of amino
acids bonded to one another by amide
groups with more than 75% of the
cystine linkages being in the form of
S-sulphocysteine, the said peptide
chains terminating at one end in a free
carboxyl group and at their other end in
a free amine group, the major part of
said chains having a molecular weight
of 1,100 to 7,500.

GB 2 115 427 A

SPECIFICATION

Keratin polymer containing S-sulphocysteine residues, process for its preparation and the compositions containing it

5 The present invention relates to a new polymer derived from keratin and containing S-sulphocysteine groups, which can be prepared by an enzymatic digestion process. 5

10 It is known that keratin polymers are of great value in the cosmetic industry for their action on the surface condition of the hair. They can be prepared by the acid or alkaline chemical hydrolysis, or preferably by the enzymatic digestion, of keratin substances. The function of the chemical or enzymatic hydrolysis is to split the long peptide chains of the keratin into water-soluble peptides of lower molecular weight. This gives mixtures of peptides of different lengths, which can contain a considerable amount of free amino acids, the peptide sequences being composed of amino acids joined to one another by amide groups and terminating at one end in a carboxyl group and at the other end in an amine group. The constituent amino acids of the 15 peptide sequences are those of which the starting keratin is composed. 15

15 The starting keratin can have a variety of origins: it can be derived, in particular, from human hair, wool, bristles, for example pig's bristles, animal hair, poultry feathers and horn. It is observed that the efficiency of the enzymatic digestion of keratin using proteolytic or keratolytic enzymes is extremely low or even zero. Thus, with the aim of improving the efficiency of enzymatic digestion, it has already been proposed to carry 20 out a sensitising treatment prior to enzymatic digestion. The efficiency of the enzymatic digestion is then substantially improved since it can be as high as 95% in some cases. 20

25 An object of the present invention is to provide a keratin polymer of novel structure, having particularly valuable characteristics in the field of hair treatment, which polymer can be prepared by enzymatic digestion having a very high efficiency. 25

25 The present invention provides a keratin polymer consisting of a statistical mixture of peptide chains of different molecular weights, derived from keratin substances and formed of amino acids bonded to one another by amide groups, the abovementioned peptide chains terminating at one end in a free carboxyl group and at their other end in a free amine group, characterised in that the peptide chains of the polymer have more than about 75% of their cystine linkages in the form of S-sulphocysteine, the major part of said 30 chains having a molecular weight from about 1,100 to about 7,500. Preferably, the major part of the peptide chains has a molecular weight from 1,300 to 4,000. The peptides of this major part having an average molecular weight of about 1,300 to about 4,000 consist of chains containing from 12 to 35 amino acids. The major part preferably represents more than 55% of the chains. 30

35 Another important characteristic of the keratin polymer according to the invention is that it has an amino acid composition, both qualitative and quantitative, which is substantially identical to that of the keratin substances from which it was prepared. By way of example, the quantitative amino acid analysis of a typical hair is compared below with that of a polymer according to the invention, prepared in accordance with the procedure of Example 1 described below. The analysis is carried out by chromatography on an ion exchange resin, after acid hydrolysis of the keratin. The results of this analysis are expressed in mols of each amino 40 acid per 100 g of total protein substance. 40

	Composition	Typical hair	Keratin polymer according to the invention	
5	Aspartic acid	4.4 10 ⁻²	4.4 10 ⁻²	5
	Threonine	5.6 "	5.7 "	
	Serine	9.8 "	9.7 "	
10	Glutamic acid	10.3 "	10.1 "	10
	Proline	6.1 "	6.3 "	
15	Glycine	4.7 "	5.1 "	15
	Alanine	3.4 "	3.6 "	
	Aminoacids derived from the cystine group	12.8 "	12.6 "	20
20	Valine	4.4 "	4.5 "	
	Methionine	0.3 "	0.3 "	
25	Isoleucine	2.1 "	2.1 "	25
	Leucine	5.0 "	5.2 "	
30	Tyrosine	1.4 "	1.2 "	30
	Phenylalanine	1.6 "	1.6 "	
	Lysine	2.0 "	2.0 "	
35	Histidine	0.6 "	0.6 "	35
	Arginine	5.2 "	5.1 "	
40	According to another characteristic of the keratin polymer according to the invention, it generally contains from 2 to 5 S-sulphocysteine residues per peptide chain. In fact, as described in detail below, in the first step of the preparation of the polymer according to the invention, substantially all the intra-chain and inter-chain cystine linkages are converted to S-sulphocysteine residues. Furthermore, knowing on the one hand that the keratin used in the preparation of the polymer according to the invention can contain about 15 cystine residues on a chain of 100 amino acids (this being the case of hair), and on the other hand that the main fraction of the keratin polymer is composed of peptides having an average of 12 to 35 amino acids, it can be seen that the peptides constituting the main fraction of the keratin polymer according to the invention contain an average of about 2 to 5 S-sulphocysteine residues per chain.	40		
45	Because of the fact that, in the preparation of the polymer according to the invention, virtually all the cystine linkages of the peptide chains of the starting keratin are converted to S-sulphocysteine residues, another characteristic of the polymer according to the invention is that it contains substantially no cysteine. This characteristic has been verified using the method described in SOKOL et al, J Soc Cosm Chem 25, 461 (1974).	45		
50	The polymer according to the invention can be presented in the form of a dry residue obtained by lyophilisation or evaporation of the solvent medium. It can also be presented in the form of an aqueous solution with a pH of at least 7. Below pH 7, the peptide chains of the polymer precipitate, but redissolve when the pH becomes alkaline again. On drying, the keratin polymer according to the invention spreads out in the form of a thin film-forming layer, which can be broken up to form shiny flakes.	50		
55	The present invention also provides a process for the preparation of a polymer of this invention from keratin substances, characterised in that, firstly, substantially all the cystine linkages between the peptide chains of the starting keratin substance are converted to S-sulphocysteine residues, the said conversion being carried out by oxidising sulphitolsis in the presence of, say, copper or thionate(s), and in that, secondly, enzymatic digestion of the keratin substance treated in this way is carried out in a manner which is in itself known.	55		
60			60	
65			65	

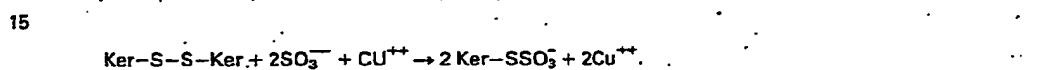
It is known that the sulphitols reaction of keratocystine is the following equilibrium reaction (in which "Ker" represents a peptide chain of the keratin):



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To displace the equilibrium of the above-mentioned reaction towards the right, so as to convert the cystine residues to S-sulphocysteine residues (Ker-SSO₃⁻), and also in order to prevent the formation of cysteine residues (Ker-S⁻), the starting keratin is treated by oxidising sulphitols. Amongst the various techniques of oxidising sulphitols which can be used in the process according to the invention, there may be mentioned, in particular, the sulphitols in the presence of copper, in an ammoniacal medium at pH 9, described by I M KOLTHOFF and W STRICKS J Amer Chem Soc, 73, 1728 (1951), the disclosure of which is hereby incorporated by reference. This sulphitols reaction can be represented as follows:

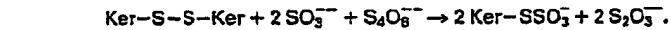
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It is also possible to conduct the oxidising sulphitols in the presence of thionate(s) as described by J L BAILEY, Biochem, J, 64-21 P (1957), the disclosure of which is hereby incorporated by reference. The sulphitols reaction in the presence of tetrathionate(s) can be represented by the following equation:

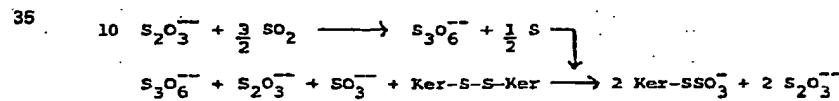
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In the process according to the invention, it is preferred to use oxidising sulphitols in the presence of trithionate. The trithionate is advantageously prepared by bubbling sulphur dioxide into a solution of thiosulphate. After removal of the precipitate of sulphur formed, the trithionate solution is brought into contact with the keratin substances to be sulphitolsed, in the presence of thiosulphate and sulphite. The two reactions for the preparation of a solution of trithionate and for the sulphitols of the cystine in the presence of trithionate can be prepared by the following scheme:

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35

40 From the practical point of view, the oxidising sulphitols should be completed by copious washing of the keratin materials treated, so as to remove the reactants. In the case of sulphitols in the presence of copper, this washing must be particularly thorough: it is generally necessary to immerse the sulphitolsed keratin substances firstly in normal hydrochloric acid at 40°C for 16 hours, and then in an equal volume of normal hydrochloric acid at ambient temperature for 24 hours. On the other hand, in the case of keratin substances treated by oxidising sulphitols in the presence of trithionate, it is simply necessary to wash them copiously with water.

40

45 The second step of the process according to the invention consists in carrying out enzymatic digestion of known type on the keratin substances treated by oxidising sulphitols, with the aid of, say, a proteolytic enzyme (such as proteinase "PSF 2019", pronase, trypsin or papain). The conditions of the enzymatic digestion, such as the pH and the enzyme/substrate ratio, naturally depend on the enzyme used.

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50 It has been found, surprisingly, that, compared with enzymatic digestion carried out in a conventional manner on keratin fibres, on the one hand the rate of enzymatic digestion in the second step of the process according to the invention, and on the other hand the extent of digestion, are substantially increased by virtue of the pretreatment of the keratin fibres by oxidising sulphitols.

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55 Thus, the extent of enzymatic digestion has been compared for:

a) natural hair;

b) the same natural hair subjected beforehand to sulphitols of a conventional type, with partial reduction of the cystine to cysteine and formation of an equivalent amount of S-sulphocysteine (as described in the article by KUNERT, ZEITSCHRIFT FÜR ALLG. MIKROBIOLOGIE, volume 16, No 2 (1976)); and

c) the same natural hair treated by means of oxidising sulphitols in the presence of copper (in accordance with the procedure described in Example 1 below).

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60 Instead of being subjected to enzymatic digestion, a fraction of the sulphitolsed hair mentioned under c) above was simply immersed in an aqueous solution at the same pH, and the degree of solubilisation of this fraction of hair d) calculated. The fractions of hair a), b) and c) above were subjected to enzymatic digestion

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carried out under the same conditions, in particular as regards the pH, the temperature and the enzyme used. Similar results to those obtained by means of oxidising sulphitolsis in the presence of copper were obtained when oxidising sulphitolsis was carried out in the presence of thionate (in accordance with the procedure described in Example 3 below) (see lines e and f of the following table). The conditions and results 5 of these comparative experiments are summarised in the following table:

	a) natural hair	- digestion with proteinase "PSF 2019" pH 9 - 24 hours - 30°C	- extent of digestion = 5%	
10	b) sulphitolsed hair	- digestion with proteinase "PSF 2019" pH 9 - 24 hours - 30°C	- extent of digestion = 15%	10
15	c) hair sulphitolsed in the presence of copper (Example 1)	- digestion with proteinase "PSF 2019" pH 9 - 2 hours - 30°C	- extent of digestion = 100%	15
20	d) hair sulphitolsed in the presence of copper (Example 1, 1st step)	- immersion in an aqueous solution pH 9 (without enzyme) - 24 hours - 30°C	- degree of solubilisation = 2%	20
25	e) hair sulphitolsed in the presence of trithionate (Example 3)	- digestion with proteinase "PSF 2019" pH 9 - 2 hours - 30°C	- extent of digestion = 100%	25
30	f) hair sulphitolsed in the presence of trithionate (Example 3, 1st step)	- immersion in an aqueous solution pH 9 (without enzyme) - 24 hours - 30°C	- degree of solubilisation = 2%	30

30 The table above clearly shows that the extent of digestion is considerably increased when the keratin fibres are subjected to a pretreatment of oxidising sulphitolsis in the presence of copper or thionate(s). It is important to note that the enzymatic digestion has virtually no effect on the proportion of S-sulphocysteine residues formed during the oxidising sulphitolsis. Thus, for the polymer prepared in accordance with the procedure described in Example 3 below, and for the initial hair used for the preparation 35 of this polymer, a quantitative analysis of the cysteine and sulphocysteine groups was carried out in accordance with the abovementioned method described by SOKOL et al. The results of this analysis are summarised in the table below.

		Cysteine	S-sulphocysteine	
40	Initial fraction of hair	0	0	40
45	Fraction of hair sulphitolsed in the presence of trithionate (according to Example 3, 1st step)	0	1.10 milliequivalents/g	45
50	Keratin polymer obtained according to Example 3	0	1.08 milliequivalents/g	50

The present invention also provides a cosmetic composition for treating the hair or skin, the said composition being characterised in that it contains, in a cosmetically acceptable carrier, an effective amount of at least one keratin polymer of this invention.

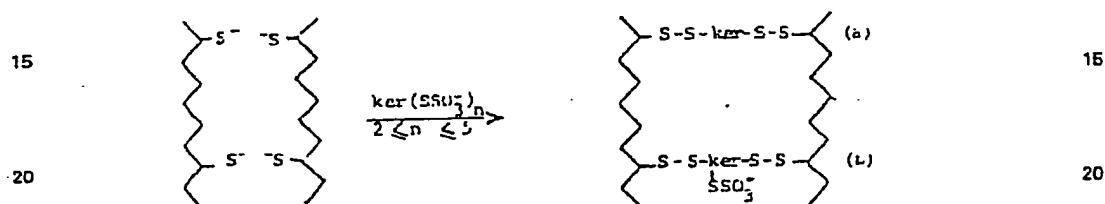
55 In fact, like all protein hydrolysates, the keratin polymer according to the invention has an affinity for the skin and keratin fibres because of the presence of free carboxyl and amine end groups at both ends of the peptide chains, which are capable of forming a large number of ionic linkages with the complementary groups of the proteins to be treated. However, the keratin polymer of the invention has a much greater affinity towards reduced keratin fibres, 60 since the S-sulphocysteine groups of the polymer (ker-S-SO₃⁻ in which "ker" represents a peptide sequence resulting from the fractionation of the long peptide chains of a keratin by enzymatic hydrolysis) react with the cysteine groups (Cys-S⁻) according to the following equilibrium reaction:



The reaction involving fixing of the cysteine by the peptides containing S-sulphocysteine groups of the polymer according to the invention is the reverse reaction of the sulphitohysis of the keratin fibres. The reaction equilibrium can be displaced towards the right if the keratin polymer is used in excess relative to the cysteine of the reduced keratin fibre.

5 Furthermore, as the peptides which constitute the main fraction of the keratin polymer contain from 2 to 5 S-sulphocysteine residues per chain, it can be seen that the fixing of the keratin polymer to the reduced fibres is accompanied by the creation of bridge linkages between the peptide chains of the reduced keratin fibres, with, moreover, an excess of reactive acid groups $-\text{SSO}_3^-$. The fixing of the polymer to the reduced keratin fibres can be represented by the following equation:

10 Reduced keratin fibre: 10 Treated keratin fibre:



15 (a) re-formation of bridges
 25 (b) re-formation of bridges and presence of a reactive acid group $-\text{SSO}_3^-$
 The "ker" group of the polymer $[\text{ker}(\text{SSO}_3^-)_n]$ represents a peptide sequence having a molecular weight of, say, 1,100 to 7,500 resulting from the fractionation of the long peptide chains of the keratin by enzymatic hydrolysis.

30 Thus, it has been observed that the fixing of the polymer according to the invention to the reduced keratin fibres has the effect of re-forming the disulphide bridges and of enriching the hair in keratin, while at the same time leaving behind reactive acid groups $-\text{SSO}_3^-$ which can be used for the fixing of, for example, basic dyestuffs, cationic compounds or alternatively one or more other cysteine radicals, the reaction thus resulting in a true crosslinking of the hair.

35 For the treatment of reduced keratin fibres, in particular hair, the treating composition used suitably contains from 1 to 10%, and preferably from 3 to 6%, by weight, of keratin polymer(s) and has a pH of at least 7 and preferably a pH of 9 to 10.

40 We have shown that if one mol of cysteine and 10 equivalents of $(\text{ker}-)-\text{SSO}_3^-$ are brought into contact at a temperature of, say, 30°C for 30 minutes at pH 9, the cysteine is "fixed" to the extent of 95% (a cysteine control at pH 9 under the same conditions, but in the absence of keratin polymer, shows a conversion of cysteine to cystine of less than 15%).

45 By analysing various reduced keratins treated with a treating composition according to the invention (containing 3% by weight of keratin polymer in solution at pH 9), we have also been able to show that the cysteine initially present in the keratin fibres disappears with time and with the number of applications.

50 Thus, if a fraction of hair is treated with a reducing liquid for soft perming, analysis shows the presence of 2% (by weight) of cysteine. The fraction of reduced hair is washed in order to remove the excess reducing agent; it is then treated at, say, 30°C with the 3% strength solution of a keratin polymer according to the invention (at pH 9). The immersion operations last 15 minutes. The hair is then towel-dried and dried at 50°C under a hood. The application of the polymer solution is repeated five times. After the third and fifth applications, the hair is rinsed with water before being dried. The proportion of cysteine in the hair during these applications is given in the following table:

	Initial fraction of hair	After the 1st application	After the 2nd application	After the 3rd application	After the 4th application	After the 5th application	
55							55
Proportion of cysteine	2%	1.1%	0.8%	0.6%	0.5%	0.4%	

60 The keratin fibres treated with the treating composition according to the invention become reactive themselves as a result of the excess S-sulphocysteine groups which remain free. The treated hair can therefore react by covalent bonding with thiols or by ionic attraction with basic dyestuffs and with cationic molecules such as trimethylcetyl ammonium bromide marketed under the name "Cetavlon" by ICI, or reactive polymers of the "ionene" type, such as those described in French Patents 2,333,012, 2,270,846, 65

2,316,271, 2,331,323, 2,331,324, 2,471,996, 2,471,776, 2,471,997 and 2,471,777. The new keratin polymer can be used as a so-called "A.P." type molecule (anionic polymer) for reaction with a molecule of the so-called "C" type (simple cationic molecule) (see JK 80 145608, 80 115813; French Patent 2,237,616; US Patents 4,247,538 and 4,210,161) or with a molecule of the so called "C.P." type (cationic polymer) (see French Patents 2,383,660 and 2,436,213) in order to impart valuable properties to the keratin fibres.

5 The following Examples further illustrate the present invention.

EXAMPLE 1

First step:

10 a) *oxidising sulphitolytic in the presence of copper*

.100 g of hair cut to a length of about 2 cm are immersed in a solution containing:

	Anhydrous Na ₂ SO ₃	350 g	
	CuCl ₂ .2H ₂ O	30 g	
15	NH ₄ OH	q.s. pH 9	15
	H ₂ O	q.s. 3.5 l	

b) *washing*

The treated hair is washed copiously with water; it is subsequently immersed for 16 hours in normal HCl at 20 40°C and then for 24 hours in normal HCl at ambient temperature. Just before enzymatic digestion, it is rinsed with water until the solution is neutral.

Second step: Enzymatic digestion

An enzymatic solution containing 1.7 g of proteinase "PSF 2019" (11,000 AU per mg, sold by ORIL, Paris) in 25 3 litres of water is brought to pH 9 with aqueous ammonia solution; the sulphitolyzed hair is added gradually to the enzyme solution. The hair is agitated continuously and the suspension is kept at a temperature of 40°C.

Every 15 minutes, the pH is readjusted to 9 with NH₄OH. After one hour, when about half of the hair has been brought into contact with the enzyme solution, a further 1.7 g of proteinase "PSF 2019" are added.

After 3 hours (from the start of the digestion operation), virtually all the hair has been digested.

30 The solution can be filtered on gauze in order to remove the residues of undigested fibres. The solution is lyophilised.

A blackish powder is obtained. The colour is due to the melanin pigments in the starting hair.

The results obtained are as follows:

35	- Extent of digestion	= 100%	
	- Initial proportion of 1/2 cystine,	1.17 milliequivalent of protein substance	35
	- Proportion of S-sulphocysteine groups	0.94 milliequivalent/g of protein substance.	

40 **EXAMPLE 2**

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First step:

a) *oxidising sulphitolytic in the presence of copper*

The same solution is used as in Example 1 a) mentioned above.

45 Immersion in the sodium sulphite/cupric chloride solution lasts for 4 hours at 60°C. The hair is agitated regularly.

b) *washing*

The same procedure is followed as in Example 1 b).

50 **50** *Second step: Enzymatic digestion*

An enzyme solution containing 7.5 g of trypsin "TPCK" (217 AU/mg) in 4 litres of water is brought to pH 8 with aqueous ammonia solution.

The sulphitolyzed hair is added gradually to the enzyme solution. The hair is agitated continuously and the suspension is kept at a temperature of 40°C.

55 Every 15 minutes, the pH is readjusted to 8 with NH₄OH. After one hour, when about 50 g of hair has been brought into contact with the enzyme solution, a further 7.5 g of trypsin are added.

After 3 hours (from the start of the digestion operation), virtually all the hair has been digested. The solution is lyophilised.

This gives a powder similar to that obtained in Example 1. The extent of digestion is about 100%.

60 **EXAMPLE 3**

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First step:

a) *oxidising sulphitolytic in the presence of trithionate*

348 g of sodium thiosulphate (Na₂S₂O₃.5H₂O) are dissolved in 3 litres of water. SO₂ is bubbled at a rate of 65 about 40 g per hour.

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The solution becomes turbid and turns yellow and a precipitate of sulphur appears. The SO_2 is allowed to evolve for 3 hours.

The precipitated sulphur is then filtered off.

A further 348 g of sodium thiosulphate are added to the solution, followed by 265 g of sodium sulphite 5 (anhydrous Na_2SO_3).

The pH is adjusted to 6.5 and the total volume is made up to 3.5 litres with water.

100 g of hair cut to a length of about 2 cm are immersed in this solution, which is kept at 40°C for 15 hours.

5

b) *washing*

10 The treated hair is washed copiously with four times 5 litres of water.

10

Second step: Enzymatic digestion

The same enzyme solution is used as in Example 1.

A black solution is obtained, which is lyophilised. The results obtained are as follows:

15 - Extent of digestion = 100 %
 - Initial proportion of $\frac{1}{2}$ cystine ... 1.17 milliequivalents/g of protein substance.
 - Proportion of S-sulphocysteine groups 1.08 milliequivalents/g of protein substance.

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20 EXAMPLE 4

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First step:

a) *oxidising sulphitolytic in the presence of trithionate*

The same solution is used as in Example 3 a) mentioned above.

25 100 g of yak hair cut to a length of about 2 cm are immersed in this solution, which is kept at 40°C for 7 hours.

25

b) *washing*

The treated yak hair is washed copiously with four times 5 litres of water.

30 *Second step: Enzymatic digestion*
 An enzyme solution containing 1.3 g of pronase in 3 litres of water is brought to pH 9 with aqueous ammonia solution. The sulphitolysed yak hair is added gradually to the enzyme solution. The hair is agitated continuously and the suspension is kept at a temperature of 40°C.

30

35 Every 15 minutes, the pH is readjusted to 9 with NH_4OH .
 After one hour, when about 50 g of hair has been added to the enzyme solution, a further 1.3 g of pronase are added.

35

After 3 hours (from the start of the digestion operation), virtually all the hair has been digested.

The solution is lyophilised. A white powder is obtained.

40 The results obtained are as follows:

40

Extent of digestion = 100 %
 - Initial proportion of $\frac{1}{2}$ cystine ... 0.83 milliequivalents/g of protein substance.
 - Proportion of S-sulphocysteine groups 0.67 milliequivalents of protein substance.

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EXAMPLE 5

First step:

a) *oxidising sulphitolytic in the presence of trithionate*

50 100 g of cut wool are immersed in a solution containing:

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- NaHSO_3 in aqueous solution (specific gravity = 1.32) 712 cm^3
 - Sodium trithionate 833 g
 - NH_4OH q.s. pH 9
 - H_2O q.s. 3.5 litres

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The immersion lasts for 15 hours at 40°C.

b) *washing*

60 The treated wool is washed copiously with four times 5 litres of water.

60

Second step: Enzymatic digestion

An enzyme solution containing 7.5 g of trypsin "TPCK" (217 AU/mg) in 4 litres of water (the same enzyme solution as in Example 2) is brought to pH 8 with aqueous ammonia solution. The sulphitolysed wool is added gradually to the enzyme solution. The wool is agitated continuously and the suspension is kept at a

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temperature of 40°C.

Every 15 minutes, the pH is readjusted to 8 with NH₄OH. After one hour, about 50 g of wool has been digested; a further 7.5 g of trypsin are then added.

After 3 hours (from the start of the digestion operation), virtually all the wool has been digested.

5 The solution is lyophilised. A white powder is obtained.

5

The results obtained are as follows:

10	- Extent of digestion	= 100 %
	- Initial proportion of 1/2 cystine ...	0.70 milliequivalents/g of protein substance.
	- Proportion of S-sulphocysteine groups	0.53 milliequivalents/g of protein substance.

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EXAMPLE 6

First step:

15 a) *oxidising sulpholysis in the presence of tritionate*

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The same solution is used as in Example 3 a) mentioned above.

100 g of red chicken feathers are immersed in this solution, which is kept at 40°C for 15 hours.

b) *washing*

16

20 The treated feathers are washed copiously with four times 5 liters of water.

20

Second step: Enzymatic digestion

The same enzyme solution is used as in Example 1 (proteinase "PSF 2019").

The digestion lasts for 3 hours at 40°C, with agitation, the pH being readjusted to 9 every 15 minutes.

25 After 3 hours, virtually all the feathers have been digested. The solution is filtered on gauze in order to remove the residues of undigested feathers. A reddish solution is obtained, which is lyophilised. The powder obtained is brown.

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The results obtained are as follows:

30	- Extent of digestion	= 90%
	- Initial proportion of 1/2 cystine ...	0.58 milliequivalent/g of protein substance.
	- Proportion of S-sulphocysteine groups	0.48 milliequivalent/g of protein substance.

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EXAMPLE 7

First step:

a) *oxidising sulpholysis in the presence of tritionate*

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The same solution is used as in Example 3 a) mentioned above.

100 g of powdered horn, washed and degreased, are immersed in this solution, which is kept at 40°C for 10 hours.

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b) *washing*

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The powdered horn is washed with several litres of water.

45 *Second step: enzymatic digestion*

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The same enzyme solution is used as in Example 4 (pronase).

The digestion is continued for 3 hours at 40°C, with agitation, the pH being readjusted to 9 every 15 minutes.

50 After 3 hours, the solution is centrifuged in order to remove the undigested particles of horn. The supernatant solution can be lyophilised.

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The results obtained are as follows:

55	- Extent of digestion	~ 75%
	- Initial proportion of 1/2 cystine ...	0.42 milliequivalent/g of protein substance.
	- Proportion of S-sulphocysteine groups	0.35 milliequivalent/g of protein substance.

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EXAMPLE 8

Improvement to the take-up of basic dyestuffs on pieces of untreated wool fabric:

60 Pieces of untreated wool fabric (5 x 4 cm) are treated by immersion for 1 hour at 40°C in a bath containing 4% of keratin polymer prepared according to Example 3, at pH 9.

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The pieces of fabric are removed from the baths and rinsed with running water. They are then immersed for 30 seconds in baths containing various dyestuff molecules, at pH 9, in an exclusively aqueous medium.

They are squeezed between two sheets of filter paper and dried for 10 minutes at 110°C.

65 By way of comparison, pieces of fabric were treated, before dyeing under the same conditions, on the one

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hand with an aqueous solution at pH 9 (NH_4OH) and on the other hand with a 4% strength solution of a commercial keratin hydrolysate not containing S-sulphocysteine groups.

The dyestuffs used are as follows:

- Methylene blue (CI 52015)
- Deorline brilliant red 3B (CI 42500)

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The intensity of the colorations is compared by a group of observers: it is noted that the pieces of fabric treated with the polymer according to the invention have taken up the dye considerably better than the others.

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EXAMPLE 9

Improvement to the take-up of basic dyestuffs on pieces of reduced wool fabric:

Pieces of wool fabric (5 x 4 cm) are treated by immersion at 40°C for 1 hour, either in a bath containing 0.1 mol/litre of thioglycolic acid at pH 9, or in a bath containing 1 mol/litre of ammonium sulphite at pH 7.

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The pieces of fabric are removed from the baths and washed copiously with water.

The washed pieces of fabric are then treated by immersion for 1 hour at 40°C in a bath containing 4% of keratin polymer prepared according to Example 4, at pH 9.

The pieces of fabric are removed from the baths and washed with running water. They are squeezed between two sheets of filter paper and are then immersed for 30 seconds in baths containing various

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dyestuff molecules at pH 9 in an aqueous medium.

They are squeezed and dried at 110°C for 10 minutes. By way of comparison, pieces of fabric were treated, after reduction and before dyeing under the same conditions, on the one hand with an aqueous solution at pH 9 (NH_4OH) and on the other hand with a 4% strength solution of a commercial keratin hydrolysate not containing S-sulphocysteine groups.

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The dyestuffs used are as follows:

- Deorline brilliant red 4G (CI 48013)
- Deorline brilliant red 3B (CI 42500)
- Astrazon blue B (CI 42140)
- Methylene blue (CI 52015)

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The intensity of the colorations is compared by a group of observers: it is noted that the pieces of fabric treated with the polymer according to the invention have fixed the dyes much better than the others. Furthermore, these pieces of fabric are more intensely coloured than those described in Example 8.

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EXAMPLE 10

Improvement to the take-up of basic dyestuffs on reduced wool fibres:

Untreated wool in the form of fibres (non-woven) is immersed for one hour at 40°C in a reducing bath containing 0.1 mol/litre of thioglycolic acid at pH 9 (NH_4OH). The wool is removed from the reducing bath; it is washed very carefully with a large amount of water.

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The reduced wool is immersed for 1 hour at 40°C in a bath containing 4% of keratin polymer prepared according to Example 3, at pH 9.

The treated wool is removed from the bath and then washed with water.

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It is then immersed for 30 seconds in baths containing basic dyestuff molecules at pH 9 in an aqueous

medium.

The wool is squeezed and then dried at 110°C for 30 minutes.

By way of comparison, wool fibres were treated, before dyeing and after reduction under the same conditions, with an aqueous solution at pH 9 (NH_4OH).

The dyestuffs used are as follows:

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- Deorline brilliant red 3B (CI 42500)
- Methylene blue (CI 52015)

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The intensity of the colorations is compared by a group of observers: it is noted that the wool treated with the polymer according to the invention is dyed much more homogeneously and that the intensity of the coloration is greater than that of the other wool samples.

EXAMPLE 11

Modification of the coloration of hair by means of basic dyestuffs:

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Three solutions, a), b) and c), are prepared for treating natural hair in order to impart a temporary coloration:

Pretreatment solution a) has the following composition:

5	- pure thioglycolic acid (depending on the final intensity of coloration desired)	0.30 g to 0.0090 g	5
	- NH ₄ OH	q.s. pH 9	
	- perfume	0.1 g	
	- water	q.s. 100 cm ³	

Treatment solution b) has the following composition:

10	- keratin polymer (according to one of the examples described)	5 g	10
	- NH ₄ OH	q.s. pH 9	
	- perfume	0.1 g	
15	- water	q.s. 100 cm ³	15

Colouring solution c) has the following composition:

20	- basic dyestuff (for example: methylene blue, doreine red 3G or astrazon blue B)	0.1 g	20
	- NH ₄ OH	q.s. pH 9	
	- perfume	0.1 g	
	- water	q.s. 100 cm ³	

25 Grey hair is treated with pretreatment solution a) for 20 minutes at 30°C. It is then washed with water. The treatment with solution b) lasts for 15 minutes at 30°C. Without washing the hair, colouring solution c) is applied for a few seconds. 25

The hair is then rinsed copiously with hot water (45°C) and dried under a hood.

The hair appears very distinctly coloured.

30 If treatment solution b) is replaced by a solution containing 5% of a keratin hydrolysate (rich in cystine) at pH 9, or 5% of gelatin at pH 9, or an aqueous solution at pH 9 (not containing the protein derivative), the hair then appears only very slightly coloured. 30

Likewise, the control hair treated directly with colouring solution c) took up the dyestuffs to only a very small extent.

35 CLAIMS 35

1. A polymeric product comprising keratin peptide chains of different molecular weights, formed of amino acids bonded to one another by amide groups, more than about 75% of the cystine linkages being in the form of S-sulphocysteine, the said peptide chains terminating at one end in a free carboxyl group and at their other end in a free amine group, the major part of said chains having a molecular weight from about 1,100 to about 7,500. 40
2. A polymeric product according to claim 1 in which the major part of the peptide chains of the polymer has a molecular weight from about 1,300 to about 4,000. 40
3. A polymeric product according to claim 1 or 2, in which its amino acid composition is substantially identical to that of the keratin substance from which it was prepared. 45
4. A polymeric product according to any one of claims 1 to 3, which contains from 2 to 5 S-sulphocysteine residues per peptide chain. 45
5. A polymeric product according to claim 1 substantially as hereinbefore described. 50
6. Process for the preparation of a polymeric product according to one of claims 1 to 5, which comprises firstly, converting substantially all the cystine linkages between the peptide chains of a keratin substance to S-sulphocysteine residues, substantially without forming cysteine residues, by oxidising sulphitolysis in the presence of copper or thionate(s), and, secondly, enzymatically digesting the resulting keratin substance. 50
7. Process according to claim 6 in which the thionate used is a trithionate. 55
8. Process according to claim 7 in which the trithionate is prepared by bubbling sulphur dioxide into a solution of thiosulphate. 55
9. Process according to any one of claims 6 to 8, in which the starting keratin substance is human hair, bristles, wool, animal hair, poultry feathers or horn. 55
10. Process according to claim 6 substantially as described in any one of Examples 1 to 7. 60
11. A polymeric product whenever prepared by a process as claimed in any one of claims 6 to 10. 60
12. A composition suitable for treating the skin or keratin fibres which comprises, in a cosmetically acceptable carrier, at least one keratin polymeric product as claimed in any one of claims 1 to 5 and 11. 60
13. A composition according to claim 12 which contains, in aqueous solution having a pH of at least 7, from 1 to 10% by weight, relative to the total weight of the composition, of the keratin polymeric product. 60

14. A composition according to claim 13 which contains, in aqueous solution having a pH of 9 or 10, from 3 to 6% by weight, relative to the total weight of the composition, of the keratin polymeric product.
15. A composition according to claim 12 substantially as described in Example 11.
16. A method of treating keratin fibres which comprises applying thereto a composition as claimed in 5 any one of claims 12 to 15.
17. A method according to claim 16 in which the keratin fibres have been treated with a reducing agent.
18. A method according to claim 17 in which the keratin fibres have been reduced on the surface, prior to the application to the said fibres of a basic dyestuff or a cationic monomer or polymer.
19. A method according to claim 16 substantially as hereinbefore described.

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